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Inheritance of Traits Related to Biological Nitrogen Fixation and Genotypic Correlation of Traits Related to Nitrogen Fixation, Yield and Drought Tolerance in Peanut (*Arachis hypogaea* L.) Under Early Drought

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Abstract: The improvement of peanut for drought tolerance and high N_2-fixation is the best way to enhance peanut production under drought condition. Besides, the heritability estimates of traits related N_2-fixation and its genetic correlation with yield and drought tolerant traits are useful to formulate the effective breeding program under drought. Therefore, the aims of this study were to estimate the heritabilities (h^2) and genotypic correlation (r_G) among traits related to N_2-fixation (TNf), yield and drought tolerant traits under early drought and non stressed condition. Ninety lines in the $F_{4:8}$ generations from four peanut crosses were tested under Field Capacity (FC) and one-third Available Water (1/3 AW). Data were recorded for Nodules Dry Weight (NDW), Biomass Production (BM), Pods Yield (PY), number of pod $plant^{-1}$, number of seed pod^{-1} and 100 seed weight at harvest. Specific Leaf Area (SLA), SPAD Chlorophyll Meter Reading (SCMR), Harvest Index (HI) and Drought Tolerance Index (DTI) of PY and BM were measured and calculated as drought tolerant traits. The h^2 for BM, PY, number of pod $plant^{-1}$ and 100 seed weight were high for all tested crosses under both water regimes. With exception of HI trait, high h^2 estimates, also, were found for drought tolerant traits under both water regimes. The genotypic correlation (r_G) between NDW and BM was positive highly significant under both 1/3 AW and FC. BM and PY showed high r_G, whereas, BM and 100 seed weight showed moderate r_G. Moderate r_G was found between BM and SCMR 60 DAE under 1/3 AW and FC. Significant correlations between FC and early drought were found for BM indicating that selection of this trait could be done under both water regimes. BM is possible to select and breed for high N_2-fixation, PY and possibly, drought tolerance because of high h^2 and significant r_G with PY and SCMR 60DAE.

Key words: *Arachis hypogaea* L., N_2-fixation, inheritance, water stress, breeding

INTRODUCTION

Peanut is an important oil seed crop due to its high nutritive value for human diet. Peanut can fix N through the symbiosis process with the help of rhizobium bacteria. This process, partly, supports to accomplish the relatively high yield of peanut and considerable one of the resources of crop's nutrient supply for poor and small-scale farmers who can not effort to use costly chemical fertilizer. Since, peanut yield is dependent on biologically N_2-fixation to a certain extent, improvement of peanut lines for their ability to fix more N may be a suitable approach for peanut yield improvement. Several traits such as nodule dry weight, biomass production, shoot dry weight, harvest index, leaf color score, pod yield and nitrogenase activity have been identified and used as selection criteria for high N_2-fixation and few studies so far have been conducted on the inheritance of these traits (Pimratch *et al*., 2004). High heritability of nitrogenase activity in many leguminous crops such as peanut was reported by Provorov and Tikhonovich (2003) and Sikinarum *et al*. (2007). Arrendrell *et al*. (1985) reported moderate to high heritability estimates for nodule number, nodule and shoot dry weight. Phudenpa *et al*. (2003) observed that, the heritability estimates for all traits related to N_2-fixation were low, especially for leaf color score in which the heritability estimates were zero or near zero in most crosses. Despite the several investigations of genetic variation and heritability for N_2-fixation related traits in peanut breeding population, estimation of heritability for these traits in particular population under irrigated and drought condition would be useful for breeders working with drought. On the other hand, doing a selection among segregated populations under adverse environmental conditions has concurrently brought water stress tolerance. These expected results may provide

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valuable information to monitoring and selecting the high N fixing genotypes under diverse environments.

Like other crops, peanut productivity can be greatly depressed by intermittent drought, which could occur at any time during the growing season when rainfall is inadequate. Also, yield can be reduced by terminal drought, which occurs when stored soil moisture is depleted resulting in crop senescence and reducing pod yield (Subbarao *et al*., 1995; Serraj *et al*., 1999). However, there is an interesting result that drought stress during pre-flowering stage can increase yield (Nageswara *et al*., 1985; Nautiyal *et al*., 1999). So, exposing peanut plant to pre-flowering drought might be a means to increase peanut productivity.

The selection and breeding of drought tolerant genotypes may help to improve the risky peanut production in drought prone environments. The breeding approach utilizing pod yield has however been unsuccessful, because, it is a quantitative trait and it showed large genotype × environment interactions (Rechards *et al*., 2001). Recently, other surrogate traits such as Transpiration Efficiency (TE) and Harvest Index (HI) have been used in drought resistance breeding program. Because of their complexity and high cost at the practical work, other easily assessable traits such as Specific Leaf Area (SLA) and SPAD Chlorophyll Meter Reading (SCMR) have become surrogate traits for TE. Samdur *et al*. (2000) and Arunyanark *et al*. (2008) reported that, the leaf chlorophyll density can be rapidly assessed using the SPAD chlorophyll meter reading and it could be used as a rapid and cost effective tool for assessment of relative chlorophyll status in peanut leaves. Since, SCMR is strongly related with SLA (Nageswara *et al*., 2001) and TE (Sheshshayee *et al*., 2006), it could be applied for indirect selection of drought resistant traits in peanut. These drought resistant traits such as SLA, SCMR, HI, Drought Tolerance Index (DTI) of biomass DTI (Biomass) and DTI (pod yield) showed high heritability estimates under both non-stressed and stressed conditions (Songsri *et al*., 2008c). In contrast, Cruickshank *et al*. (2004) reported that broad-sense heritability of transpiration (T), TE and HI were varied among peanut crosses and traits depending on levels of genetic variation in peanut.

There are two possible ways to formulate the development of peanut productivity under drought; (1) improvement of genotypes with high ability of N_2-fixation and (2) breeding of drought resistant genotypes. Both of them are crucially important to obtain the expected high yield. Information of the heritability and the genetic correlations among traits related to N_2-fixation, yield and drought tolerant traits will provide the essential guideline for peanut breeders. Perhaps, drought can alter the inheritance of the interested traits, thus, their heritability should be estimated under different water regimes to clarify the effect of early drought upon the genetic variation and heritability estimates. To our knowledge, no heritability estimates of traits related to N_2-fixation under early season drought and no phenotypic and genotypic correlations for these traits with yield and drought tolerant traits in the literature.

Therefore, the aims of the current study were to estimate the heritability of traits related to N_2-fixation and genotypic correlation among traits related to N_2-fixation, yield and drought tolerant traits under early drought.

MATERIALS AND METHODS

Plant materials: The experiment was conducted at Khon Kaen University's Field Crop Research Station during December 2007 to April 2008. F_1 generations of four peanut crosses (ICGV 98300×KK 60-3, ICGV 98300×Tainan 9, ICGV 98303×Tainan 9 and ICGV 98305×Tainan 9) were generated from the hybridization of 3 drought resistant lines (ICGV 98300, ICGV 98303 and ICGV 98305) selected for low yield reduction with two high yielding cultivars KK 60-3 and Tainan 9. ICGV lines and Tainan 9 have medium seed, whereas, KK 60-3 has large seed and the maturity of ICGV lines, KK 60-3 and Tainan 9 are 110, 120 and 100 days, respectively. KK 60-3 is a released cultivar commonly grown in Thailand. It is a Virginia-type peanut cultivar with high N_2-fixation (Toomsan *et al*., 1995) but sensitive to drought for pod yield. The F_1 seeds were harvested in bulk for each cross. In F_2 and F_3 generations, two pods were kept for each plant and bulk for each cross. Line separation was carried out in the F_4 generation. A total of 90 lines (25 lines each of first and second crosses and 20 lines each of third and fourth crosses) were randomly selected and they were multiplied in the F_6 and F_7 generations.

The 90 families from 4 crosses were evaluated in the $F_{4:8}$ generations (F_4-derived lines in the F_8 generations, respectively) under two soil moisture levels {field capacity (FC) and 1/3 available soil water (1/3 AW)} in dry season of 2007/08. A split-plot design with four replications was used and plot size was a five rows plot with 3 m long with spacing of 20 cm between plants within row and 40 cm between rows.

Crop management: The land was plowed three times before planting. Lime at the rate of 625 kg ha^{-1} was incorporated into the soil during soil preparation.

Phosphorous fertilizer as triple superphosphate at the rate of 24.7 kg P ha^{-1} and potassium fertilizer as muriate of potash (KCl) at the rate of 31.1 kg K ha^{-1} were applied as basal dose before planting. Seeds were treated with a fungicide (Captan) at the rate of 5 g kg^{-1} of seed before planting. Three seeds were planted per hill and thinning was done to obtain one plant per hill at 21 days after planting (DAP). Pre-emergence weed control was carried out by spraying alachlor at the rate of 3 L ha^{-1} soon after planting and hand weeding was followed at 15 and 35 DAP.

Rhizobium inoculation was done by applying a water-diluted commercial peat-based inoculum of *Bradyrhizobium* (mixture of strains THA 201 and THA 205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) on the rows of peanut plants. Gypsum ($CaSO_4$) was applied at the rate of 312 kg ha^{-1} at the pod filling stage. Pest and disease were controlled by weekly applications of Carbosulfan at 2.5 L ha^{-1}, methomyl at 1.0 kg ha^{-1} and carboxin at 1.68 kg ha^{-1}. Carbofuran 3% granular was applied at the pod setting stage by side dressing.

Irrigation: Subsurface drip irrigation system was installed to supply water to the peanut plants and soil water level was maintained uniformly at field capacity from planting to 7 DAP in both FC and water stressed plots. The soil moisture of water stress plot decreased gradually and from then on it was maintained to lead at the level of 1/3 AW ±1% constantly of the pre-determined level until flowering (40 DAE). Water was added to the respective plots by subsurface drip irrigation based on the crop water requirement and surface evaporation which were calculated following the methods described by Songsri *et al*. (2008a).

The total crop water use for each water treatment was calculated as the sum of transpiration and soil evaporation. Transpiration (T) was calculated using the methods described by Doorenbos and Pruitt (1992) as follows:

$$\text{ET crop} = \text{ETo} \times \text{Kc}$$

where, ETcrop: crop water requirement (mm/day), ETo: evapotranspiration of a reference under specified conditions calculated by pan evaporation method, Kc: the crop water requirement coefficient for peanut, which varies with genotypes and growth stage. In addition to, surface evaporation (Es) was calculated using the following formula which described by Singh *et al.* (1993):

$$\text{Es} = \beta \times (\text{Eo}/\text{t})$$

where, Es is soil evaporation (mm), β is light transmission coefficient measured depending on crop cover, Eo is evaporation from class A pan (mm day^{-1}) and t is days from the last irrigation or rain (day).

DATA COLLECTION

Weather parameters: The experiment was conducted during the dry season, December 2007 to April 2008. The maximum rainfall was 23.4 mm at 110 DAE during experiment established (data not shown). The maximum and minimum air temperature were 31.7 and 19.7°C, respectively. In addition to, daily pan evaporation ranged from 1.78 to 12.68 mm and the solar radiation was 18.0 MJ m^{-2} day^{-1} as a seasonal mean.

Soil moisture status: Soil moisture in the individual plots was measured by gravimetric soil analysis at planting and harvest at the depths of 0-5, 25-30 and 55-60 cm at 0, 10, 25, 40 and 50 DAP to calculate the required amount of water to apply. The soil water status was also weekly monitored with a neutron moisture meter (Type I.H. II SER. N° N0152, Ambe Diccot Instruments Co. Ltd., England) at a depth of 0.3 to 0.6 m at 0.3 m intervals.

Traits related to biological N_2-fixation, yield and yield components: Data were recorded for nodule dry weight and biomass production as N_2-fixation parameters at harvest only. Five plants from each plot were randomly chosen and were carefully dug to recover as many nodules as possible. The plants were cut at ground level to separate roots from shoots. The samples were washed with tap water and nodules were removed from roots by hand. The nodules were oven dried at 80°C for 48 h and the dry nodule was weighed.

Two kilogram random sample of shoots was oven-dried at 80°C for 48 h and dry weight was measured. Pods were air-dried to obtain approximately 8% moisture content and shelled. Then, pod number $plant^{-1}$, seed number pod^{-1}, pod yield, 100 seed weight and harvest index were determined. To estimate the top biomass production with pods, bordered plants in an area of 3.36 m^2 were harvested from each plot, then, the pods were taken off and the roots were cut. The shoots were oven-dried at 75°C for 48 h and dry weight was measured. Biomass production was calculated by combining the dry weight of shoot and pod.

Drought tolerant traits: Specific Leaf Area (SLA) and SPAD Chlorophyll Meter Reading (SCMR) were measured

Table 1: Analysis of variance of cross and cross product

		Mean square of character				
Source of variation	Degree of freedom	X	Y	MCP†	EMS‡	EMCP§
Replication	r-1					
Genotypes (G)	g-1	M_2*	M_2	$M*_2 M_2$	$\sigma^2_E + r\sigma^2_F$	$\sigma_{E*E} + r\sigma_{F*F}$
Error	(r-1)(g-1)	M_1*	M_1	$M*_1 M_1$	σ^2_E	σ_{E*E}

† MCP: Mean square of cross product, ‡ EMS: Expected mean square, § EMCP: Expected mean square of cross product

at 20, 40, 50 and 60 days after emergence. The third fully-expanded leaves from their respective terminal bud were detached from 5 chosen plants which were randomly selected from each plot between 8:30 and 9:00 am. SPAD chlorophyll reading was recorded twice on each leaflet of the tetra foliate leaf along the mid-rib. The model of this SPAD chlorophyll meter reading device was Minolta SPAD-502 m, Tokyo, Japan. So, Nageswara *et al*. (2001) suggested to fully covering the SPAD meter sensor upon the leaf lamina to avoid the interference from veins and midribs. SLA was measured on these leaves after recording SCMR at the same day. Measuring the leaf area with a leaf area meter (LI 3100C Area Meter, LI-COR Inc., USA) was followed by drying the leaves in an oven at 80°C for at least 48 h and weighted. SLA was calculated using the following formula:

$$\text{SLA} = \text{Leaf area (cm}^2\text{)/Leaf dry weight (g)}$$

Harvest index was computed as the ratio of total pods weight to total biomass at the final harvest. Drought tolerance indexes (DTI) of pod yield (PY) and biomass production were calculated using Songsri *et al*. (2008b) formula as follow;

$$\text{DTI (PY)} = \frac{\text{Pod yield under stressed treatment}}{\text{Pod yield under well-watered condition}}$$

Data analysis: The data were subjected to analysis of variance according to a split plot design. In case of significant difference, mean comparison was flowed based on Duncan's multiple range test (Gomez and Gomez, 1984). Despite collecting SLA and SCMR data on 20, 40, 50 and 60 DAE, the data with the lowest CV and the highest F ratio were considered in data interpretation for precision. SLA data at 50 DAE and SCMR data at 60 DAE were focused to analysis of the variances.

Estimates of broad-sense heritability for 4 crosses were calculated by the formula described by Holland *et al*. (2003) as follow:

$$h^2 = \sigma^2_G/\sigma^2_P$$

$$h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2/r)$$

Where:

h^2 = Broad-sense heritability

σ^2_G = Genotypic variation

σ^2_P = Phenotypic variation

σ^2 = Error mean square

r = No. of replication

The Standard Error (SE) of heritability (Singh *et al*., 1993) for traits related to N_2-fixation was calculated to give a measure of the precision of the estimate. Since, the evaluation of heritability estimates was conducted in late generations (F_8) of segregating materials when most genes were nearly fixed in individual genotypes, it would be expected that additive genetic variances for the traits under study were purified through generation advance (Holland *et al*., 2003).

Phenotypic and genotypic correlation coefficients among traits related to N_2-fixation, pod yield, yield components and drought tolerant traits were calculated based on genotypic means using the following methods which summarized in (Table 1) and described by Falconer and Mackay (1996):

$$\text{Phenotypic correlation } (r_P) = (M*_2 M_2)/[(M_2*)(M_2)]^{½}$$

$$\text{Genotypic correlation } (r_G) = (M*_2 M_2 - M*_1 M_1) / [(M_2* - M_1*)(M_2 - M_1)]^{½}$$

The value of M_1, M_2, M_1* and M_2* were calculated based on the analysis of variance of cross and cross product (Table 1). Simple correlation was used to determine the relationship between nodule dry weight and biomass production under irrigated and early season drought conditions to understand whether the performance of peanut genotypes was consistent across environments or not.

RESULT AND DISCUSSION

Soil moisture content: Soil moisture was weekly measured using a neutron moisture meter until harvest to directly check whether the water treatments were correct enough or not because water supply was calculated based on weather data. A clear distinction between two soil moisture levels noted at 30 cm of soil depth showed

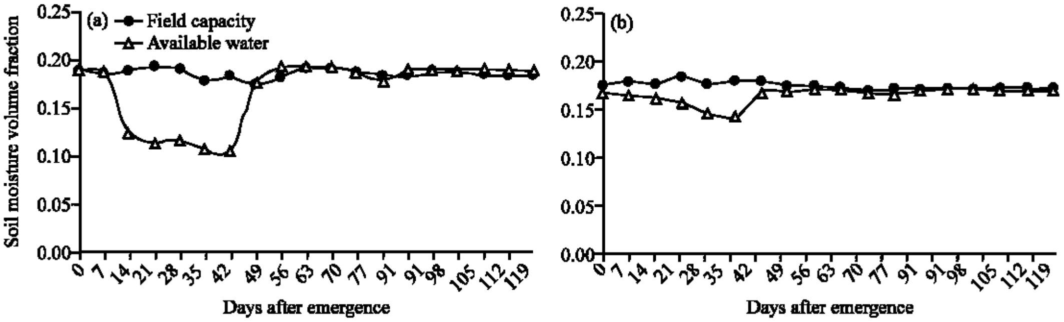

Fig. 1: Soil moisture volume fraction in two available soil water regimes [field capacity (FC) and 1/3 available water (AW)] at 30 (a) and 60 cm (b) of the soil depths during 2007/08 dry season

Table 2: Progenies means for all 4 peanut crosses of 90 lines under early season drought in the dry season of 2007/08

Traits	ICGV 98300×KK 60-3	ICGV 98300×Tainan 9	ICGV 98303×Tainan 9	ICGV 98305×Tainan 9
Traits related to N_2-fixation				
NDW	1.11b	1.20b	1.44a	1.26b
BM	9.71a	7.46b	7.33b	7.66b
Drought tolerance traits				
SLA 50 DAE	166.23b	160.72b	163.41b	177.94a
SCMR 60 DAE	44.99a	44.46a	41.65b	41.82b
HI	0.32ab	0.32ab	0.31b	0.33a
DTI (BM)	1.15ab	1.18ab	1.13b	1.21a
DTI (PY)	1.33b	1.53a	1.24b	1.33b
Yield component traits				
PY	3.98a	2.58b	2.51b	2.61b
No. of pod plant^{-1}	28.68a	26.50b	20.82d	22.45c
No. of seed pod^{-1}	1.89a	1.85a	1.85a	1.88a
100 seed weight	56.21a	37.28d	48.39b	44.57c

Different letter(s) in each row of each parameters show significant at $p<0.05$ by Least Significant Difference test (LSD). NDW: Nodule Dry Weight, BM: Biomass Production, SLA: Specific Leaf Area, SCMR: SPAD Chlorophyll Meter Reading, DAE: Day after Emergence, HI: Harvest Index, DTI: Drought Tolerance Index, PY: Pod Yield

Table 3: Progenies means for all 4 peanut crosses of 90 lines under irrigated condition in the dry season of 2007/08

Traits	ICGV 98300×KK 60-3	ICGV 98300×Tainan 9	ICGV 98303×Tainan 9	ICGV 98305×Tainan 9
Traits related to nitrogen fixation				
NDW	1.13b	1.09b	1.43a	1.34a
BM	8.76a	6.39b	6.65b	6.46b
Drought tolerance traits				
SLA 50 DAE	198.79b	212.30a	197.56b	192.63c
SCMR 60 DAE	42.89a	40.95b	40.04c	39.96c
HI	0.32a	0.28c	0.30b	0.31ab
Yield component traits				
PY	3.24a	1.76c	2.10b	2.05b
No. of pod plant^{-1}	23.40a	22.28a	17.61c	19.84b
No. of seed pod^{-1}	1.88a	1.84a	1.78b	1.85a
100 seed weight	51.54a	36.14c	46.12b	44.30b

Different letter(s) in each row of each parameters show significant at $p<0.05$ by Least Significant Difference test (LSD). NDW: Nodule Dry Weight, BM: Biomass Production, SLA: Specific Leaf Area, SCMR: SPAD Chlorophyll Meter Reading, DAE: Day after Emergence, HI: Harvest Index, DTI: Drought Tolerance Index, PY: Pod Yield

that soil moisture could be controlled along crop development to treat with the desired moisture levels (Fig. 1a-b). The soil moisture difference due to 2 treatments under study was smaller at 60 cm depth. Since, there was not any significant change in the weather data and no interference of rain during drought treatment, water-regime treatment was carried out correctly until harvest.

Mean performances: The mean performances of study traits revealed that ICGV 98303×Tainan 9 cross had higher means for nodules dry weight, whilst, ICGV 983000×KK 60-3 cross had higher means for biomass production under both water regimes (Table 2, 3). In addition to, ICGV 98300×KK 60-3 cross was high in means of most drought tolerant traits such as SCMR 60 DAE, HI and DTI (BM) under early drought. This cross also had the highest means of SCMR 60 DAE and HI under irrigated condition (Table 2, 3). Surprisingly, this cross showed the highest means of pod yield and yield components under both stressed and well-watered conditions. Needless to doubt, the parental line; ICGV 98300 is drought tolerant and high yielding line, while the parental line; KK 60-3 is high in N_2-fixation (Toomsan *et al*., 1995). On the other hand, ICGV 98303×Tainan 9 cross showed lower means of drought tolerant traits, pod yield and yield components under both stressed and irrigated conditions although it had the high N_2- fixation. Lower means of pod yield were found in ICGV 98300×Tainan 9 and ICGV 98305×Tainan 9 crosses but their means of N_2-fixation related traits were quite high (Table 2, 3).

Heritability of traits related to N_2-fixation, pod yield, yield components and drought tolerant traits: Analysis of variance followed the earlier studies of Arrendrell *et al*. (1985), Pimratch *et al*. (2004) and Sikinarum *et al*. (2007) who reported that genotypes were the main source of significant variation ($p<0.01$) for all N_2-fixation related traits, all agronomic and drought tolerant traits, confirming the presence of variability in the genetic materials (data not shown). Heritability estimates within 4 peanut crosses were calculated for nodule dry weight and biomass production at harvest and shown in Table 4. Heritability estimates for nodule dry weight were low in all crosses under both water regimes and ranged from 0.05 to 0.32. Similarly, Pimratch *et al*. (2004) found low heritability in nodule dry weight (0.00-0.40) of their experimental genotypes, whereas, high heritability estimate for nodule dry weight (0.63-1.00) was reported by Sikinarum *et al*. (2007). The opposite results might be, due to differences in experimental condition and used materials. Present result indicated that, the improvement for these traits will be difficult with least possibility. Besides, heritability of each crosses were consistent under both water regimes showing that early drought could not alter their genetic expression in nodule dry weight. Nigam *et al*. (1985) reported that additive genetic variance was more important than non-additive genetic variance for nodule dry weight. In contrast, Miller *et al*. (1986) reported that, the importance of non-additive genetic variance was greater than additive genetic variance for nodule dry weight. However, selection will be effective, only, when heritability estimate was high and additive genetic effect is greater. Although, ICGV 98303×Tainan 9 cross had low heritability estimates for nodule dry weight, its performance for this trait was high (Table 2). This might be due to high performance of this trait of both parents (high×high). Therefore, improvement for agronomic traits might be possible in this cross if heritability estimates were high.

All crosses showed high heritability estimates for biomass production under both early drought and irrigated conditions as shown in Table 4 and ranged from 0.73 to 0.89. This range was relatively narrow for heritability estimates of biomass and it was a stable factor in this population. Moreover, these similarities between two water regimes showed that, it was possible to select and breed for N_2-fixation using biomass production trait as effective and easy selection tool.

Despite relating to N_2-fixation, generally, the heritability of nodule dry weight was poorer than that of biomass production under two water regimes. In term of practical work, measuring biomass production data is several times easier than that of nodule dry weight. This useful information of high heritability estimates of biomass production and its consistency under early drought and irrigated condition gave a certain extent to achieve the breeding progress for this character.

Table 4: Estimates of heritability with standard error for traits related to N_2-fixation; nodule dry weight (NDW), biomass production (BM) and yield component traits; pod yield (PY), number of pod plant^{-1}, number of seed pod^{-1} and 100 seed weight at harvest of 4 crosses of peanut under Early Season Drought (ESD) and irrigated conditions

	Heritability					
	Traits related to N_2-fixation			Yield component traits		
Crosses	NDW	BM	PY	No. of pod plant^{-1}	No. of seed pod^{-1}	100 seed weight
ESD						
ICGV 98300×KK60-3	0.23±0.05	0.88±0.04	0.84±0.05	0.75±0.07	0.46±0.09	0.87±0.04
ICGV 98300×Tainan 9	0.05±0.02	0.88±0.04	0.58±0.09	0.79±0.06	0.38±0.09	0.93±0.03
ICGV 98303×Tainan 9	0.32±0.07	0.83±0.05	0.80±0.05	0.65±0.08	0.32±0.07	0.93±0.02
ICGV 98305×Tainan 9	0.26±0.11	0.73±0.07	0.74±0.06	0.56±0.08	0.54±0.08	0.94±0.02
Irrigated						
ICGV 98300×KK60-3	0.22±0.06	0.89±0.04	0.86±0.04	0.91±0.03	0.24±0.07	0.79±0.06
ICGV 98300×Tainan 9	0.05±0.02	0.78±0.06	0.88±0.04	0.79±0.06	0.08±0.03	0.87±0.04
ICGV 98303×Tainan 9	0.16±0.33	0.87±0.04	0.85±0.04	0.56±0.08	0.21±0.06	0.85±0.04
ICGV 98305×Tainan 9	0.27±0.11	0.88±0.04	0.88±0.03	0.87±0.04	0.69±0.07	0.93±0.02

ESD: Early Season Drought, NDW: Nodule Dry Weight, BM: Biomass Production, PY: Pod Yield

Table 5: Estimates of heritability with standard error for Specific Leaf Area (SLA) at 50 Days after Emergence (DAE), SPAD Chlorophyll Meter Reading (SCMR) at 60 DAE, Harvest Index (HI) and Drought Tolerance Index of Biomass Production (DTI (BM)) and Pod Yield (DTI (PY)) of 4 crosses of peanut under Early Season Drought (ESD) and irrigated conditions

	Heritability				
	Drought tolerant traits				
Crosses	SLA 50 DAE	SCMR 60DAE	HI	DTI (BM)	DTI (PY)
ESD					
ICGV 98300×KK60-3	0.94±0.02	0.89±0.04	0.19±0.06	0.76±0.07	0.66±0.08
ICGV 98300×Tainan 9	0.87±0.04	0.85±0.05	0.24±0.11	0.71±0.08	0.80±0.06
ICGV 98303×Tainan 9	0.93±0.02	0.83±0.05	0.58±0.08	0.64±0.08	0.67±0.07
ICGV 98305×Tainan 9	0.86±0.04	0.92±0.02	0.07±0.03	0.75±0.06	0.69±0.07
Irrigated					
ICGV 98300×KK60-3	0.75±0.07	0.81±0.06	0.32±0.08	-	-
ICGV 98300×Tainan 9	0.87±0.04	0.80±0.06	0.60±0.09	-	-
ICGV 98303×Tainan 9	0.90±0.03	0.91±0.03	0.70±0.07	-	-
ICGV 98305×Tainan 9	0.85±0.04	0.90±0.03	0.44±0.08	-	-

†DTI were calculated by the ratio of ESD (1/3 Available Water (AW))/non-stressed (Field Capacity (FC)) conditions. ESD: Early Season Drought, SLA: Specific Leaf Area, SCMR: SPAD Chlorophyll Meter Reading, DAE: Day After Emergence, HI: Harvest Index, DTI: Drought Tolerance Index

Most pod yield and yield component traits had similar heritability estimates except number of seed pod^{-1} when they were compared between two water regimes. All crosses showed high heritability estimates for pod yield, number of pod plant^{-1} and 100 seed weight for different water regimes. Heritability estimates for pod yield and number of pod plant^{-1} were moderate to high but widely ranged from 0.58 to 0.88 and 0.56 to 0.91, respectively (Table 4). Also, heritability estimates for 100 seed weight were high and ranged from 0.79 to 0.94, whereas, for number of seed pod^{-1} were moderate in most crosses and quite low (0.08) in ICGV 98300×Tainan 9 cross (Table 4). Present results were not in agreement with Kesmala *et al.* (2004) who reported that estimates of heritability for most agronomic traits were consistently low. Variable results have been reported in other earlier studies concerning to estimation of heritability for agronomic traits in peanut. Nonetheless, the relatively high and moderate heritability estimate for yield and yield component traits in this study were supposed to favor selection in later generations.

Moreover, heritability estimates within four peanut crosses were calculated for HI, SLA 50 DAE, SCMR 60 DAE, DTI (BM) and DTI (PY) (Table 5). HI, DTI (BM) and DTI (PY) were calculated after harvest only. Different heritability estimates were found for HI within four crosses under both water regimes, ranging from 0.07 to 0.70. Variation in HI might be affected by early drought because plant tried to resist water stress by drought tolerant mechanism (Taiz and Zeiger, 2006) and fixed nitrogen might provide to vegetative parts rather than yield under drought (Wunna *et al*., 2009). HI had different heritability estimates because this trait was a portion of pod yield and it was a quantitative trait with various genetic expressions within peanut crosses. In addition to, other drought tolerant traits such as SLA 50 DAE, SCMR 60 DAE, DTI (BM) and DTI (PY) showed high heritability estimates for four peanut crosses under both water stressed and non-stressed conditions, ranged from 0.64 to 0.94 (Table 5). These results were in agreement with Songsri *et al.* (2008c) who found that estimates of heritability for drought resistant traits were consistently high under both non-stressed and stressed conditions. Early drought could not alter the heritability estimates of such traits.

Genotypic correlation between traits related to N_2-fixation: Phenotypic correlation is not reported because both phenotypic and genotypic correlation showed the same information in this study. Higher genotypic correlation was found between nodule dry weight and biomass production under early drought (0.77, $p \leq 0.01$) than irrigated condition (0.65, $p \leq 0.01$) (Table 6). Similarly, Pimratch *et al.* (2004) reported that there was high correlation among fixed N, nodule dry weight, shoot dry weight and total dry weight. Fixed nitrogen generally contributed to vegetative growth rather than to yield. Pimratch *et al.* (2008b) reported that correlation between fixed N and biomass production was higher under drought compared to well-watered conditions. The result implied that under early drought peanut nodules might provide fixed nitrogen to nodule development and plant growth. Under drought condition, it was so hard to mine soil nitrogen in drying soil by roots that there was the inability of peanut to use soil nitrogen effectively (Osman *et al*., 1983) and peanut depended only on biological N_2-fixation. In addition, both traits were measured at harvest only and it reduced the precise with which their genotypic correlation was measured.

Genotypic correlation between traits related to N_2-fixation and yield and yield components: Nodule dry weight had negative and significant correlation with pod

Table 6: Genotypic (r_G) correlation estimates between traits related to N_2-fixation, drought resistant traits and yield component traits for all 4 peanut crosses of 90 lines under Early Season Drought (ESD) and irrigated condition (degree of freedom = 356)

	Traits related to N_2-fixation	
Traits	NDW	BM
ESD		
Traits related to N_2-fixation		
NDW		0.77**
BM	0.77**	
Drought tolerance traits		
SLA 50 DAE	-0.15**	0.25**
SCMR 60 DAE	0.15**	0.57**
HI	0.47**	0.36**
DTI (BM)	-0.15**	0.16**
DTI (PY)	0.10*	0.05
Yield components		
PY	-0.75**	0.79**
No. of pod plant^{-1}	-0.70**	0.47**
No. of seed pod^{-1}	-0.79**	0.41**
100 seed weight	-0.15**	0.52**
Irrigated		
Traits related to nitrogen fixation		
NDW		0.65**
BM	0.65**	
Drought tolerance traits		
SLA 50 DAE	-0.22**	-0.41**
SCMR 60 DAE	-0.07	0.44**
HI	0.01	0.29**
Yield components		
PY	0.10*	0.68**
No. of pod plant^{-1}	0.05	0.44**
No. of seed pod^{-1}	0.09	0.22**
100 seed weight	0.08	0.49**

* and ** significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. DTI were calculated by the ratio of ESD (1/3 AW)/irrigated (FC) conditions. ESD: Early Season Drought, NDW: Nodule Dry Weight, BM: Biomass Production, SLA: Specific Leaf Area, SCMR: SPAD Chlorophyll Meter Reading, DAE: Day after Emergence, HI: Harvest Index, DTI: Drought Tolerance Index, PY: Pod Yield

yield and yield components under early drought, ranging from (-0.15** to -0.75**) (Table 6). But their correlation was not significant under irrigated condition. Likewise, Pimratch *et al.* (2004) reported that, there was not any correlation among N_2-fixation parameters and agronomic traits except for 100 seed weight. Present results indicated that under early drought, fixed nitrogen might be beneficial to nodules growth and other vegetative parts rather than pod yield.

On the other hand, biomass production was highly correlated with pod yield under early drought and irrigated condition, 0.79** and 0.68**, respectively. The correlation was higher under early drought because fixed nitrogen supplied to plant growth under drought and this advantage contributed to yield after re-watering. Moderate correlation was found between biomass production and number of pod plant^{-1} (0.47**), number of seed pod^{-1} (0.41**) and 100 seed weight (0.52**) under stressed condition (Table 6). Under well-watered condition, the correlation coefficients between biomass production and number of pod plant^{-1}, number of seed pod^{-1} and 100 seed weight were 0.44**, 0.22** and 0.49**, respectively. Between these two traits related to N_2-fixation, biomass production had higher correlation with pod yield and yield components. Progenies means of biomass production and pod yield were generally higher under early drought than well-watered condition (data not shown). These results strongly supported a consumption that fixed nitrogen might be more partitioning to vegetative growth under early drought and the stronger in plant growth in turn contributed to be higher in pod yield. After early drought, peanut plants might need a certain time to recover and then, fixed nitrogen contributed to 100 seed weight by more translocation during seed filling stage. This increase in yield resulting from decreased irrigation in the early stages of a crop's life may be exploited in crop management when irrigation is available (Nageswara *et al.*, 1985). Moreover, these correlations were stable under different water regimes, it would be possible to improve pod yield by selecting of high biomass production in peanut genotypes.

Genotypic correlation between traits related to N_2-fixation and drought tolerant traits: The correlations of nodule dry weight and biomass production with SCMR, SLA and HI were examined in order to better understand these relationships and to assess whether SCMR and SLA could be used as selection tools to enhance N_2-fixation. Nodule dry weight was negatively correlated with SLA at 50 days after emergence (50 DAE) under early drought and irrigated condition, -0.15** and -0.22**, respectively (Table 6). Both N_2-fixation and leaf area were affected by drought (Taiz and Zeiger, 2006) but Sikinarum *et al.* (2007) found that total dry matter accumulation was independent of root nodule or rate of fixation. Therefore, nodule dry weight, otherwise, N_2-fixation was not correlated with SLA because leaf dry weight is a part of SLA. The correlation between nodule dry weight and SCMR 60DAE was low (0.15**) under water stress and negative (-0.07) under irrigated condition (Table 6). As a matter of fact, drought reduced the nitrogen fixation (Hungria and Vargas, 2000; Giller, 2001; Pimratch *et al.*, 2008a, b) and increased the chlorophyll density (Nageswara and Wright, 1994; Arunyanark *et al.*, 2008). The higher chlorophyll density under early drought might provide more photosynthate to other plant growth rather than nodules. This assumption became more obvious with another correlation between nodule dry weight and HI. It was moderate (0.47**) under early drought but very low

Table 7: Correlation coefficients of nodule dry weight (NDW) and biomass (BIO) at harvest of 4 peanut crosses under early season drought (d) and irrigated (w) conditions (degree of freedom (df) = 18 for ICGV 98300×KK 60-3, ICGV 98300×Tainan 9 and df = 23 for ICGV 98303×KK 60-3 and ICGV 98305×KK 60-3)

	Peanut cross			
Correlation	ICGV 98300×KK 60-3	ICGV 98300×Tainan 9	ICGV 98303×Tainan 9	ICGV 98305×Tainan 9
NDW_w vs NDW_d	0.04	0.03	0.13	-0.14**
BIO_w vs BIO_d	0.31**	0.24**	0.37**	0.18**

**Significant at $p \le 0.01$

(0.01) under irrigated condition. Similarly, Pimratch *et al.* (2004) reported that N_2-fixation parameters were negatively correlated with HI under irrigated condition. So, present result indicated that fixed nitrogen contributed to reproductive growth under well-watered condition. Moreover, its correlation with DTI (PY) was quite low (0.10*) whereas its correlation with DTI (BM) was negative (-0.15**) (Table 6).

Furthermore, other N_2-fixation parameter, biomass production was not highly correlated (0.25**) with SLA 50 DAE under early drought and it showed a negative correlation of (-0.41**) under irrigation. Leaf area reduction is the first sign of drought response by plant (Taiz and Zeiger, 2006) but fixed nitrogen might go to biomass production under drought condition. Therefore, their correlations were not highly significant. Nonetheless, its correlation to SCMR 60 DAE was moderate under early drought and irrigated condition, 0.57** and 0.44**, respectively (Table 6). In contrast, high genotypic correlation was reported between biomass production and SCMR under both drought and irrigated condition by Songsri *et al.* (2008c). Therefore, it can be concluded that higher chlorophyll density under early drought might support the biomass production instead of partitioning to sink. Their correlation was so consistent that selection is possible for either fixed nitrogen or drought resistance. Present results did not strongly support the above conclusion, because, their correlation was just moderate. HI was moderately correlated with biomass production under early drought and irrigated condition, 0.36** and 0.29**, respectively (Table 6). It needs not to doubt because as we mentioned before, biomass production was one fraction of HI. But its correlation to other drought tolerant traits such as DTI (BM) and DTI (PY) were quite low and negative (Table 6).

Relationship of traits related to N_2-fixation under early drought versus irrigated condition: Biomass production was possible to be selected under either early drought or irrigated condition, because, biomass production under both water regimes showed a significant correlation in all four peanut crosses (Table 7). Whereas, nodule dry weight showed low correlation values in three crosses of four crosses under study and negative correlation value in remain cross (ICGV 98305×Tainan 9). These correlations of biomass production revealed that more suitable condition for selecting these traits related to N_2-fixation could be done under both well watered and water stressed conditions. A fairly good correlation will favor to select the particular trait under all conditions. In addition, since the heritability estimates of biomass production were high in all peanut crosses under both early drought and irrigated conditions, selection of biomass production is a better way to select high nitrogen fixed genotypes among the tested populations.

CONCLUSION

Peanut lines derived from cross of ICGV 98300×KK 60-3 are recommended because of their high ability to maintain high N_2-fixation, high drought tolerance and high contribution to pod yield under early drought and field capacity. Early drought can not alter the heritability estimates of nodule dry weight and biomass production. It can rather increase the pod yield of some peanut genotypes which can maintain high N_2-fixation under early drought. Based on heritability estimates, biomass production showed a high possibility to improve N_2-fixation among four crosses of peanut population. It may be useful as a selection criterion for high N_2-fixation and pod yield because of, its high genotypic correlation with pod yield and yield components in all 4 crosses. Despite its moderate correlation with SCMR, it would lead to a proposal that SCMR may be an alternative tool of detecting fixed nitrogen to a certain extent in a particular population. Since, measuring nodule dry weight incurs a substantial cost with low heritability and it has low correlation with pod yield and drought tolerant traits, biomass production is possible to select and breed for higher N_2-fixation under early drought and well-watered conditions.

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REFERENCES

Arrendrell, S., J.C. Wynne, G.H. Elkan and T.G. Isleib, 1985. Variation for nitrogen fixation among progenies of a Virginia × Spanish peanut cross. Crop Sci., 25: 865-869.

Arunyanark, A., S. Jogloy, C. Akkasaeng, N. Vorasoot, T. Kesmala, R.C. Nageswara Rao, G.C. Wright and A. Patanothai, 2008. Chlorophyll stability is an indicator of drought tolerance in peanut. J. Agron. Crop Sci., 194: 113-125.

Cruickshank, A., A. Dowkiw, G. Wright, N. Rachaputi and S. Nigam, 2004. Heritability of drought-resistance traits in peanut. Proceedings of the 4th International Crop Science Congress, Sept. 26-Oct. 1, Brisbane, Australia, pp: 1-2.

Doorenbos, J. and W.O. Pruitt, 1992. Calculation of Crop Water Requirements. In: Crop Water Requirements. FAO Irrigation and Drainage Paper No. 24, Rome, Italy, pp: 1-65.

Falconer, D.S. and T.F.C. Mackay, 1996. Introduction to Quantitative Genetics. 4th Edn., Benjamin Cummings, England, ISBN-10: 0582243025.

Giller, K.E., 2001. Nitrogen Fixation in Tropical Cropping Systems. 2nd Edn., CAB International, Wallingford, UK., ISBN-10: 0851986714.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. 2nd Edn., John Wiley and Sons, Inc., New York, ISBN-10: 0471870927.

Holland, J.B., W.E. Nyquist and C.T. Cervantes-Martinez, 2003. Estimating and interpreting heritability for plant breeding: An update Plant Breed. Rev., 22: 9-111.

Hungria, M. and M.A.T. Vargas, 2000. Environmental factors affecting N_2 fixation in grain legumes in the tropics, with an emphasis on Brazil. Field Crop Res., 65: 151-164.

Kesmala, T., S. Jogloy, S. Wongkaew, C. Akkasaeng, N. Vorasoot and A. Patanothai, 2004. Heritability and phenotypic correlation of resistance to *Peanut bud necrosis virus* (PBNV) and agronomic traits in peanut. Songklanakarin J. Sci. Technol., 26: 129-138.

Miller, Jr., J.C., K.W. Zary and G.C.J. Fernandez, 1986. Inheritance of N fixation efficiency in cowpea. Euphytica, 35: 551-560.

Nageswara, R.R.C., S. Singh, M.V.K. Sivakumar, K.L. Srivastava and J.H. Williams, 1985. Effect of water deficit at different growth phase of peanut. I Yield response. Agron. J., 77: 782-786.

Nageswara, R.R.C. and G.C. Wright, 1994. Stability of the relationship between specific leaf area and carbon isotope discrimination across environments in peanut. Crop Sci., 34: 98-103.

Nageswara, R.R.C., H.S. Talwar and G.C. Wright, 2001. Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypogaea* L.) using chlorophyll meter. J. Agron. Crop Sci., 189: 175-182.

Nautiyal, P.C., V. Ravinda, P.V. Zala and Y.C. Joshi, 1999. Enhancement of yield in peanut following the imposition of transient soil-moisture-deficit stress during the vegetative phase. Exp. Agric., 35: 371-385.

Nigam, S.N., S.L. Dwivedi, P.T.C. Nambiar, R.W. Gibbons and P.J. Dart, 1985. Combining ability analysis of N_2-fixation and related traits in peanut. Peanut Sci., 12: 55-57.

Osman, A.K., J.C. Wynne, G.H. Elkan and T.J. Schneeweis, 1983. Effect of removal on symbiotic nitrogen fixation in peanut. Peanut Sci., 10: 107-110.

Phudenpa, A., S. Jogloy, B. Toomsan, S. Wongkaew, T. Kesmala and A. Patanothai, 2003. Heritability and phenotypic correlations of traits related to N_2-fixation and agronomic traits in peanut (*Arachis hypogaea* L.). Songklanakarin J. Sci. Technol., 26: 317-325.

Pimratch, S., S. Jogloy, B. Toomsan, P. Jaisil, J. Sikhinarum, T. Kesmala and P. Patanothai, 2004. Evaluation of seven peanut genotypes for nitrogen fixation and agronomic traits. Songklanakarin J. Sci. Technol., 26: 295-304.

Pimratch, S., S. Jogloy, N. Vorasoot, B. Toomsan, T. Kesmala, A. Patanothai and C.C. Holbrook, 2008a. Effect of drought stress on traits related to N_2 fixation in eleven peanut (*Arachis hypogaea* L.) genotypes differing in degrees of resistance to drought. Asian J. Plant Sci., 7: 334-342.

Pimratch, S., S. Jogloy, N. Vorasoot, B. Toomsan, A. Patanothai and C.C. Holbrook, 2008b. Relationship between biomass production and nitrogen fixation under drought stress conditions in peanut genotypes with different levels of drought resistance. J. Agron. Crop Sci., 194: 15-25.

Provorov, N.A. and I.A. Tikhonovich, 2003. Genetic resources for improving nitrogen fixation in legume-rhizobium symbiosis. Genet. Resour. Crop Evol., 50: 89-99.

Rechards, R.A., A.G. Condon and G.J. Rebetzke, 2001. Traits to Improve Yield in Dry Environments. In: Application of physiology in Wheat Breeding, Reynolds, M.P., I.J. Ortiz-Monasterio and A. McNab (Eds.). CIMMYT, Mexico, pp: 89–100.

Samdur, M.Y., A.L. Singh, R.K. Mathur, P. Manivel, B.M. ChiKani, H.K. Gor and M.A. Khan, 2000. Field evaluation of chlorophyll meter for screening groundnut (*Arachis hypogaea* L.) genotype tolerant to iron deficiency chlorosis. Curr. Sci., 79: 211-214.

Serraj, R., T.R. Sinclair and L.C. Purcell, 1999. Symbiotic N_2 fixation response to drought. J. Exp. Bot., 50: 143-155.

Sheshshayee, M.S., H. Bindumadhava, N.R. Rachaputi, T.G. Prasad, M. Udayakumar, G.C. Wright and S.N. Nigam, 2006. Leaf chlorophyll concentration relates to transpiration efficiency in peanut. Ann. Applied Biol., 148: 7-15.

Sikinarum, J., P. Jaisil, S. Jogloy, B. Toomsan, T. Kesmala and A. Patanothai, 2007. Heritability and correlation for nitrogen (N2) fixation and related traits in peanut (*Arachis hypogaea* L.). Pak. J. Biol. Sci., 10: 1956-1962.

Singh, M., S. Ceccarelli and J. Hamblin, 1993. Estimation of heritability from varietal trials data. Theor. Applied Genet., 86: 437-441.

Songsri, P., S. Jogloy, N. Vorasoot, C. Akkasaeng, A. Patanothai and C.C. Holbrook, 2008a. Root distribution of drought-resistant peanut genotypes in response to drought. J. Agron. Crop Sci., 194: 92-103.

Songsri, P., S. Jogloy, T. Kesmala, N. Vorasoot, C. Akkasaeng, A. Patanothai and C.C. Holbrook, 2008b. Heritability of drought resistance traits and correlation of drought resistance and agronomic traits in peanut. Crop Sci., 48: 2245-2253.

Songsri, P., S. Jogloy, T. Kesmala, N. Vorasoot, C. Akkasaeng, A. Patanothai and C.C. Holbrook, 2008c. Response of reproductive characters of drought resistant peanut genotypes to drought. Asian J. Plant Sci., 7: 427-439.

Subbarao, G.V., C. Johansen, A.E. Slinkard, R.C. Nageswara Rao, N.P. Saxena and Y.S. Chauhan, 1995. Strategies for improving drought resistance in grain legumes. Critic. Rev. Plant Sci., 12: 469-523.

Taiz, L. and E. Zeiger, 2006. Stress Physiology. In: Plant Physiology, Taiz, L. and E. Zeiger (Eds.). Sinauer Associates, Inc., Sunderland, MA., pp: 671-681.

Toomsan, B., J.F. McDonagh, V. Limpinuntana and K.E. Giller, 1995. Nitrogen fixation by groundnut and soybean and residual nitrogen benefits to rice in farmers' fields in Northeast Thailand. Plant Soil, 175: 45-56.

Wunna, H., S. Jogloy, B. Toomsan and J. Sanitchon, 2009. Response to early drought for traits related to nitrogen fixation and their correlation to yield and drought tolerance traits in peanut (*Arachis hypogaea* L.). Asian J. Plant Sci., 8: 138-145.